Effect of atrial natriuretic factor on transmural myocardial blood flow distribution in the dog

ROBERT P. BAUMAN, M.D., JUDITH C. REMBERT, PH.D., STEVEN I. Himmelstein, M.D., PAUL E. KLOTMAN, M.D., AND JOSEPH C. GREENFIELD, JR., M.D.

ABSTRACT These studies were designed to define the effect of atrial natriuretic peptide (ANP) on coronary flow. ANP was infused as a bolus directly into the left circumflex coronary artery in doses ranging from 0.05 to 5 μg in nine open-chest, anesthetized dogs. Coronary flow was measured with an electromagnetic flowmeter. Regional transmural myocardial blood flow and distribution were measured with 11.3 ± 0.25 μm radionuclide-labeled microspheres. No significant change was noted in systemic hemodynamics (heart rate, arterial pressure, left atrial pressure, or cardiac output) during the course of the studies. ANP produced a transient vasodilatation of coronary resistance vessels and increased flow by 41% after both the 2.5 and 5 μg doses. The vasodilatation occurred uniformly throughout the ventricular wall so that the endocardial/epicardial flow ratio remained constant. There was no evidence of coronary vasoconstriction. The peak vasodilatation response occurred 28 ± 7 sec after the beginning of the infusion of ANP and lasted approximately 3 min. These data support the hypothesis that ANP administration is associated with a vasodilator response in the coronary resistance vessels that may be modulated through either the release of another vasodilator substance or another mechanism.


SYSTEMIC ADMINISTRATION of atrial natriuretic peptide (ANP) has been shown to produce a number of hemodynamic effects, including a slight reduction in arterial pressure and cardiac output. Generally, administration of ANP results in slight increases in flow to specific organs. The effects of ANP on myocardial blood flow are somewhat controversial. Wangler et al., using an isolated Langendorf-perfused guinea pig heart noted marked coronary vasoconstriction after infusion of atriopeptin II. These authors postulated that this vasoconstrictor effect might result in reduced cardiac function. On the other hand, Garcia et al. found that synthetic ANP produced a marked increase in myocardial blood flow measured with 15 μm microspheres in conscious rats. Recently, Bache et al. noted a reduction in coronary vascular resistance after infusion of ANP into the canine coronary circulation during constant blood perfusion. So far as we are aware, there are no data available that define the effects of ANP on transmural myocardial blood flow distribution. Accordingly, the present study was designed to determine the effects of ANP on the canine coronary circulation. Atrial peptide 1-28 (Peninsula Labs, Inc.) was infused directly into the left circumflex coronary artery while flow in this vessel was measured with an electromagnetic flowmeter and myocardial blood flow was determined with 11.3 ± 0.25 μm radionuclide-labeled microspheres. This route of administration enabled assessment of the direct effect of ANP on the canine coronary circulation without secondary effects resulting from changes in other hemodynamic variables that might be associated with systemic infusion.

Methods

Data were obtained on nine adult mongrel dogs (weight 25 to 30 kg). Each was anesthetized with 45 mg of morphine given intramuscularly followed in 30 min by 80 mg/kg α-chloralose given intravenously over a 45 min period. Supplemental chloralose anesthesia was given as needed during the procedure. These studies were carried out under a protocol approved by the Animal Experimentation Committees of Duke Medical Center and the Durham Veterans Administration Medical Center. The dogs were intubated, maintained on a Harvard respirator, and the heart of each was exposed via a left thoracotomy. The left atrium and the ascending aorta were cannulated with 50 cm long polyvinyl chloride catheters (1.0 mm inside diameter) via a small
pulmonary vein and internal mammary artery, respectively. The left circumflex coronary artery was dissected minimally so that a Howell electromagnetic flowmeter probe (EMF), 2.5 to 3.5 mm, could be placed just under the left atrial appendage and stabilized. Infusions were made into the left circumflex coronary artery by inserting a 26-gauge short beveled atraumatic needle directly into the coronary artery just proximal to the EMF probe. To reduce the possibility of streaming of the drug resulting in inhomogeneous perfusion, care was taken to orient the needle perpendicularly to the vessel and the injection of the drug was made rapidly. Intravascular pressures were measured by connecting the catheters to P23 dB Gould-Statham transducers and Hewlett-Packard Model 8805B amplifiers. The EMF probes were calibrated both from the measured resistivity of the probes and by passing known volumes of normal saline through the probes in a given period of time.

After the instrumentation was in place, phasic and mean left atrial and arterial pressures along with phasic and mean left circumflex coronary arterial blood flow and the electrocardiogram were recorded on a direct-writing oscillograph (Hewlett-Packard model 8800) and FM magnetic tape. These hemodynamic variables were recorded continuously throughout the experiment. Myocardial blood flow and cardiac output were measured by injecting 11.3 ± 0.25 μm microspheres labeled with one of seven different radioactive nuclides (New England Nuclear) into the left atrium. With the level of myocardial blood flow found in our study, this would result in approximately 1200 microspheres/g section of myocardium.

The ANP used in these experiments was diluted in normal saline so that 0.05, 0.5, 2.5, and 5 μg had a final volume of 0.5 ml. In addition, 0.05 mg of adenosine was diluted in 0.5 ml of normal saline.

The experimental protocol was as follows. All hemodynamic variables were recorded continuously. Myocardial blood flow determinations were made with the use of randomly chosen microspheres after 0.5 ml infusions of normal saline (used as control) and 0.05, 0.5, 2.5, and 5 μg of ANP and 0.05 mg of adenosine infused sequentially into the circumflex coronary artery. The duration of the intracoronary infusion was 2 to 5 sec. The microspheres were injected approximately 20 sec after the infusion of ANP was started. Our preliminary data with ANP had indicated that peak flow would occur at approximately 25 sec after the infusion was begun. Thus, the microspheres were circulated at a time in which peak flow occurred and in which a reasonably steady state in flow was noted. At least 15 min was allowed between each infusion.

After the experimental protocol had been completed, each animal was killed with intravenous sodium thiopental and intravenous potassium chloride. Methylene blue was infused at the same time into the circumflex coronary artery to stain the area of myocardium perfused by the circumflex coronary artery. The heart and 1 g samples of leg muscle, diaphragm, kidney, liver, and spleen were excised and fixed in 10% buffered formalin. Two days later the heart was cut into four transverse rings and each ring was sectioned into seven circumferential regions: anterior septal, anterior, anterior papillary, lateral, posterior papillary, posterior, and septal. Each region was cut further into four transmural layers of approximately equal thickness with layer 1 the epicardial layer and layer 4 the endocardial layer. The resulting sample weights ranged from 0.3 to 1.5 g.

The left and right atria were stripped of all connective tissue and cut into regions. The techniques for cutting the left ventricle, counting the samples, and calculating myocardial blood flow in our laboratory have been described in detail previously and therefore will be only briefly outlined here. However, the method we use to measure cardiac output with microspheres has not been defined previously.

The mathematical relationship for determining blood flow with the microsphere technique is:

$$Q_i/Q_c = C_i/C_r$$

where $C_i$ is the total number of counts present in the blood reference samples that were obtained during the microsphere injection and $Q_i$ is the rate of withdrawal of these blood samples from the arterial catheter via a calibrated withdrawal pump. $C_r$ is the total number of counts in the tissue sample, and $Q_c$ is the unknown tissue blood flow. By rearranging this relationship as:

$$Q_c = Q_i \times (C_r/C_i)$$

the total blood flow to the tissue sample can be estimated for each isotope injected. The total tissue sample blood flow is divided by the corresponding tissue sample weight and expressed as blood flow in milliliters per minute per gram of tissue. The endocardial/epicardial blood flow ratio is determined by dividing the blood flow to the innermost layer by the blood flow to the outermost layer, i.e., layer 4 divided by layer 1.

A similar relationship is used for determining cardiac output ($Q_{co}$):

$$Q_{co} = Q_i \times (C_i/C_r)$$

where $C_{co}$ and $Q_c$ are the same as for the tissue blood flow calculation. $C_i$ is the total amount of activity injected into the left atrium and is determined in the following manner for each isotope. Several drops of the appropriate microsphere suspension are placed in preweighed plastic counting vials just before injection. These are reweighed, and the weight of microsphere suspension is determined by subtracting the weight of the empty vial. The empty syringe from the microsphere injection and the plastic vials are counted in the gamma spectrometer for 10 min each. An estimate of the counts per milligram of microsphere suspension is obtained by dividing the total number of counts in each vial by the weight of the microsphere suspension in that vial. An average of the counts per millgram of microsphere suspension for the vials is determined. The difference between the full and empty syringe weights is multiplied by the average counts per milligram to obtain an estimate of the total number of counts contained in the syringe before injection. The total number of counts remaining in the empty syringe is subtracted from this value to determine $C_{co}$, the total number of counts injected into the left atrium.

Two techniques were used to determine whether a given section of myocardium was perfused with ANP. The area perfused by the circumflex was stained with methylene blue. In addition, after the flow in the samples was computed, only areas of ventricular and atrial myocardium that showed both a marked increase in flow after infusion of adenosine and were stained with methylene blue were considered to be infused by the ANP. Control samples consisted of those areas that were not stained with methylene blue and showed no evidence of increased blood flow after infusion of adenosine. The region of the ventricle infused by ANP was either the lateral or posterior papillary regions and the control region was either the anterior or anterior papillary region. The atrial regions infused by ANP were most frequently the lateral wall of the left atria and posterior wall of the right atria. The roofs of both left and right atria were most frequently control regions.

Hemodynamic data were measured directly from the oscillographic recording. These included heart rate, mean and phasic arterial and left atrial pressures, and mean and phasic circumflex coronary flow. The time to achieve peak flow as well as total duration of the increase in flow were measured from the beginning of injection. Data are presented as mean ± SD. Comparisons of hemodynamic and myocardial blood flow data between the different pertubations were analyzed by Student’s $t$ test and
the p value was corrected for similar comparisons by the Bonferroni method.

**Results**

The hemodynamic data are listed in table 1. Immediately before, during, and after each bolus, arterial pressure, left atrial pressure, and heart rate were found not to change significantly throughout the period of study. Infusions of saline (control) and each dose of ANP as well as adenosine were not associated with significant changes in any of the measured hemodynamic variables. Cardiac output did not change significantly during the entire study.

Review of the EMF tracings indicated that the infusion of ANP was associated with a slight but inconsistent increase in circumflex coronary flow after both the 0.05 and 0.5 μg doses. Both the 2.5 and 5.0 μg doses of ANP elicited an increase in coronary flow. The 2.5 μg dose resulted in a change in flow from the control value of 38 ± 4 to 48 ± 6 ml/min. The peak flow occurred 28 ± 7 sec from the beginning of the bolus infusion. This response lasted for approximately 3 min. None of the infusions of ANP were associated with either transient or sustained reductions in coronary flow. Adenosine elicited a much larger increase in peak flow, from a control value of 32 ± 8 ml/min to a maximum flow of 100 ± 21 ml/min, which occurred 18 ± 3 sec from the beginning of the bolus infusion. The adenosine peak flow response occurred significantly earlier than the ANP peak flow response (p < .05).

The myocardial blood flow data are listed in table 2. Mean transmural flow and the endocardial/epicardial ratio for the circumflex bed (ANP perfused region) are listed in rows 1 and 2 and the mean transmural flow and endocardial/epicardial ratio for the left anterior descending bed (control region) are listed in rows 3 and 4. After both the 0.05 and 0.5 μg dose of ANP there was a trend for myocardial blood flow to increase; however, these changes did not reach statistical significance. After the 2.5 μg dose of ANP, flow increased by a mean of 41% from a control value of 0.92 ± 0.29 to 1.30 ± 0.37 ml/min/g (p < .05). A similar increase in myocardial blood flow was noted after the 5.0 μg infusion of ANP. Infusion of adenosine was associated with an average change in flow of 191%. In the control

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>Saline (control)</th>
<th>Dose of ANP</th>
<th>Adenosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>90±26</td>
<td>90±27</td>
<td>105±26</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>99±20</td>
<td>103±21</td>
<td>107±16</td>
</tr>
<tr>
<td>Left atrial pressure (mm Hg)</td>
<td>5.4±4.1</td>
<td>4.7±2.0</td>
<td>4.9±2.1</td>
</tr>
<tr>
<td>Cardiac output (liters/min)</td>
<td>2.31±0.87</td>
<td>2.73±1.39</td>
<td>2.68±0.25</td>
</tr>
</tbody>
</table>

No significant (p < .05) change from control occurred in any of the hemodynamic variables during the course of the study. Data are mean ± SD.

**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th>Saline (control)</th>
<th>Dose of ANP</th>
<th>Adenosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circumflex region (ANP)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean flow</td>
<td>0.92±0.29</td>
<td>1.11±0.36</td>
<td>1.22±0.16</td>
</tr>
<tr>
<td>Endo/epi</td>
<td>1.04±0.33</td>
<td>0.92±0.19</td>
<td>1.05±0.33</td>
</tr>
<tr>
<td>Left anterior descending region (control)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean flow</td>
<td>0.76±0.28</td>
<td>0.89±0.30</td>
<td>0.90±0.15</td>
</tr>
<tr>
<td>Endo/epi</td>
<td>0.99±0.18</td>
<td>0.99±0.17</td>
<td>1.05±0.14</td>
</tr>
<tr>
<td>Atrial ANP region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean flow</td>
<td>0.49±0.16</td>
<td>0.63±0.11</td>
<td>0.57±0.17</td>
</tr>
<tr>
<td>Atrial control region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean flow</td>
<td>0.46±0.13</td>
<td>0.57±0.19</td>
<td>0.52±0.21</td>
</tr>
</tbody>
</table>

Myocardial blood flow is given in ml/min/g, mean ± SD.

<sup>A</sup>p < .05 compared with saline (control) within each region.

<sup>B</sup>p < .05 compared with control regions for each intervention.
region flow did not change significantly throughout the study. The endocardial/epicardial ratio in both the ANP and control regions did not differ significantly from unity and did not change significantly throughout the study.

Adequate samples of atrial tissue that met the conditions for perfusion by ANP were obtained in only four dogs (table 2), and thus the results are somewhat tenuous. Although there was a trend for flow to increase with all doses of ANP, only the 5 μg dose of ANP was associated with a significant change from control value (0.49 ± 0.16 to 0.69 ± 0.10 ml/min/g). Adenosine infusion in this same region was associated with an average increase in flow of 283%. Flow to the atrial control regions (table 2) demonstrated no significant change throughout the period of the study. The mean flow to the other organs sampled (skeletal muscle, diaphragm, liver, spleen, and kidney) did not change significantly throughout the course of the study.

**Discussion**

The preparation chosen in which ANP was infused directly into one of the coronary arteries allowed us to elucidate the direct effect on myocardial blood flow secondary to ANP without any concomitant changes in any of the other hemodynamic variables. Results indicate that under the conditions of the experiment there was no evidence of transient myocardial vasoconstriction. The infusion of ANP in both the 2.5 and 5 μg doses was associated with a 41% increase in mean transmural flow that was distributed equally throughout the four layers of the myocardium so that the endocardial/epicardial ratio remained constant. Thus, there was no preferential vasodilatation in any layer of the myocardium. These results are different from those of Wangler et al.,3 who noted a marked vasoconstriction associated with the infusion of atropeptin II, but are consistent with the findings of both Garcia et al.2 and Bache et al.4 One potential problem with infusion of a drug directly into the coronary artery is that layering or streaming of the drug may occur so that the perfusion of the myocardium is inhomogeneous. If this occurs, spurious data could result. Care was taken to infuse the drug with a small needle, which should result in turbulence at the needle tip and obviate streaming. If streaming had occurred, one might expect a wide variation among the dogs in the endocardial-to-epicardial flow ratio; however, this was not found. In addition, 150 μg of ANP was given into the left atrium in four dogs and blood flow distribution was measured. In these dogs an average of 37% increase in transmural blood flow occurred and the mean endocardial/epicar-
dial ratio (0.98) remained essentially unchanged. An average decrease in mean arterial pressure of 13 mm Hg was associated with this infusion of ANP. These data indicate that in a situation in which streaming of the drug could not occur, a similar result in transmural blood flow distribution was obtained.

The time course of the vasodilator response to infusions of ANP was different than that of the response to adenosine; it took 28 ± 7 sec for the maximum vasodilation to occur with ANP, whereas adenosine was associated with a much more marked vasodilator response, which occurred significantly earlier (18 ± 3 sec). (p < .05). These data are consistent with the hypothesis that ANP may not have the same direct vasodilator action as adenosine on resistance vessels. ANP may have actions secondary to release of another vasodilator substance or through another mechanism. The increased blood flow associated with ANP was quite evanescent, lasting only 3 min, and represents vasodilation of the resistance vessels of the coronary bed. Whether or not ANP affected the large coronary arteries, i.e., capacitance vessels, could not be elucidated. However, since ANP has been shown to relax smooth muscle and these large muscular arteries contain considerable smooth muscle, it is quite likely that ANP also dilates the large coronary arteries. A relatively large amount of ANP was infused directly into the coronary bed. For example, with the measured values of cardiac output, myocardial blood flow, and weight of myocardium perfused by ANP, it can be calculated that the 5 μg dose is similar to a dose of approximately 250 μg (or 8 μg/kg) infused as a bolus into the left atrium.

The results of these studies indicating a brief vasodilatation of the coronary artery resistance vessels after infusion of ANP are consistent with previous studies reported by Garcia et al.2 and Bache et al.4 Percent change in coronary flow, i.e., vasodilator response, was similar to that noted by Bache et al.4 These investigators did note significant reduction in coronary resistance at a much lower dose than in the present studies, perhaps as a result of a fixed flow preparation. However, no evidence of even a transient vasoconstriction due to ANP was noted in our studies. Thus, our data do not support the conclusion that a reduction in cardiac function secondary to coronary vasoconstriction may result from infusions of ANP. Why the responses of the coronary circulation in our study are the opposite of those described by Wangler et al.3 is unclear, but could be due to differences in the animal preparation or to differences in the atrial peptides used. Lappe et al.7 demonstrated differences in the hemodynamic re-
sponse to atriopeptin II and rat ANP in various vascular beds of the spontaneously hypertensive rat. However, in their study, both of these agents always produced a vasodilator response in each of the vascular beds investigated.

In our previous study, submaximal vasodilating doses of adenosine were found to produce a significant preferential increase in flow to the endocardial layer as manifest by an endocardial/epicardial ratio markedly greater than 1. In the present studies, the endocardial/epicardial flow ratio was not different from unity. The explanation for the differences in these findings is undoubtedly related to differences in the methods used. In our prior study, infusions of adenosine were given in varying doses to produce a specific increase in flow and the amount of adenosine varied markedly between dogs. In the present study, adenosine was given only to indicate the area that was subsequently infused by ANP and a standard dose of 0.05 mg of adenosine was given. Thus, as expected, the flow after adenosine varied markedly from 1.18 to 4.0 ml/min/g and the endocardial/epicardial ratio varied from 0.66 to 1.98. The higher flows in which maximum vasodilation occurred were found to result in lowered endocardial/epicardial ratio, as noted in our previous studies, whereas the lower flows resulted in endocardial/epi-


cardial ratio greater than unity. The mean endocardial/epicardial ratio for the group was not significantly different from 1.

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